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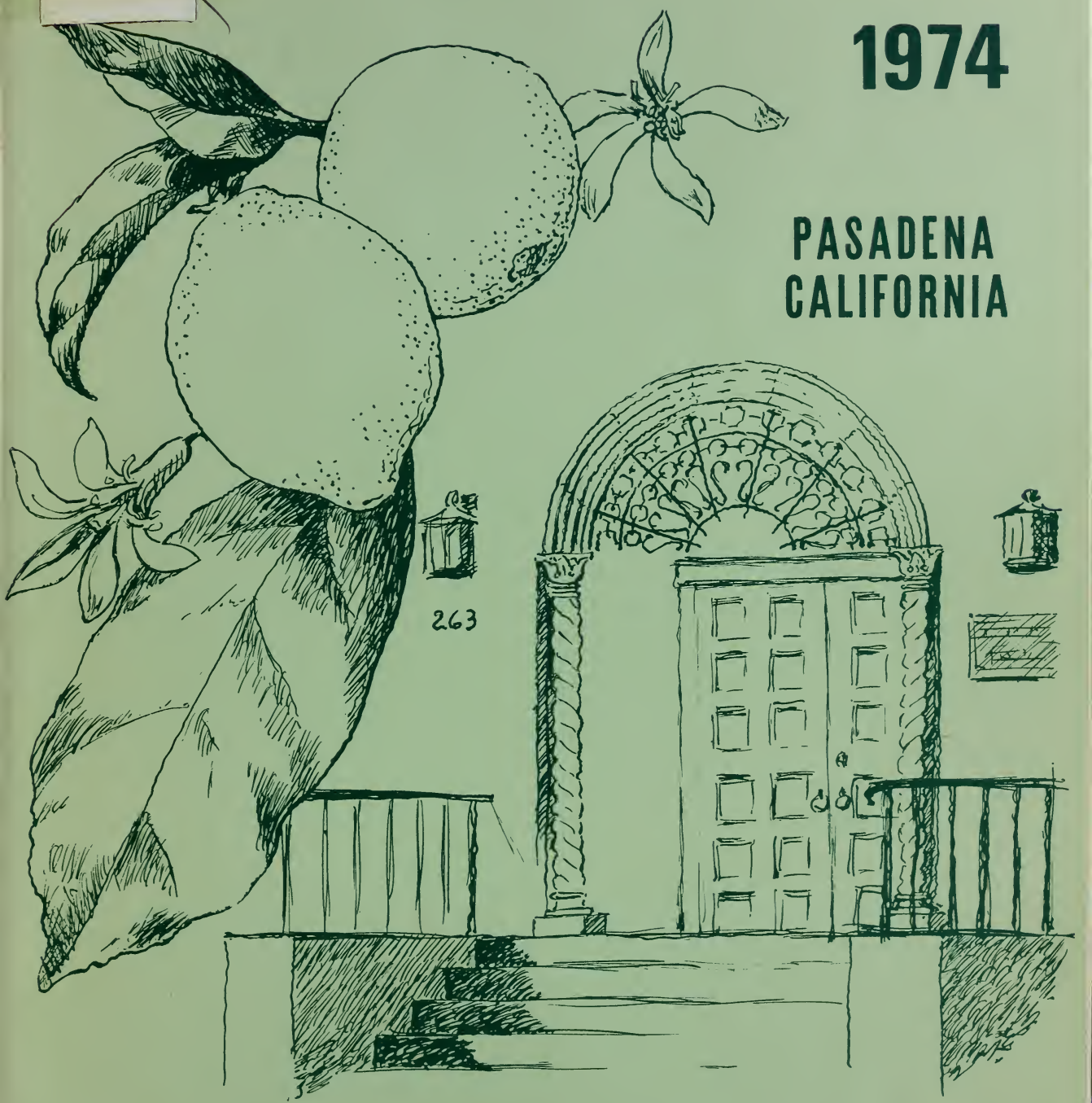

CITRUS RESEARCH CONFERENCE

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December 11, 1974

Pasadena, California

ABSTRACTS OF PAPERS

Sponsored By:

Fruit and Vegetable Chemistry Laboratory
263 South Chester Avenue
Pasadena, California 91106

Agricultural Research Service
UNITED STATES DEPARTMENT OF AGRICULTURE

FOREWORD

This Citrus Research Conference is being held to bring to members of the citrus and allied industries in southern California and Arizona the latest results of research on the chemistry, pharmacology, and technology of citrus fruits and their products carried on by the Agricultural Research Service, U.S. Department of Agriculture. The following are participating in this year's conference.

Western Region

Fruit and Vegetable Chemistry Laboratory
263 South Chester Avenue, Pasadena, California 91106

Southern Region

Citrus and Subtropical Products Laboratory
600 Avenue S, N.W., Winter Haven, Florida 33882

Food Crops Utilization Research Laboratory
P.O. Box 388, Weslaco, Texas 78596

Conference headquarters:

Huntington-Sheraton Hotel
1401 South Oak Knoll Avenue
Pasadena, California 91109

PROGRAM

CITRUS RESEARCH CONFERENCE

Wednesday, December 11, 1974

MORNING SESSION - 9:00 A.M.

L U N C H

AFTERNOON SESSION - 1:45 P.M.

INVESTIGATIONS INTO THE CHEMICAL NATURE OF LEMON JUICE CLOUD*

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Cloud stability is one of the important factors determining quality of citrus juices, and a considerable amount of research has been carried out in this area. However, the chemical nature of the cloud particles has received relatively little attention. We have recently begun a study of the chemical composition of lemon juice cloud, with special emphasis on its surface chemical properties. Here we report some initial results of this investigation.

Reconstituted juice from a commercial concentrate and fresh juice, prepared by hand reaming in the laboratory, were the experimental materials. Cloud was isolated for general chemical analysis by centrifuging at high speed. The pellet thus obtained was lyophilized and extracted with organic solvents. The insoluble residue was then assayed for protein, pectin, and nucleic acid content. Corrections were made for juice solubles occluded in the pellet. In general, results for the concentrate and fresh juice were quite similar. In both cases about half of the cloud was soluble in organic solvents. This soluble fraction was a complex mixture which included fatty acid esters, sterols, phospholipids, and flavonoid glycosides. The remaining insoluble fraction was composed largely of pectin and protein.

For studies of the surface chemistry of cloud particles, isolation by centrifuging was unsatisfactory, because the cloud pellet could not be resuspended without changing its properties. An ultrafiltration apparatus provided a solution to this problem of removing soluble material from the cloud. The juice, in a stirred cell, was forced through a membrane filter by air pressure. Water was added to maintain the original volume, until almost all of the juice solubles had been washed through the filter. During this process the cloud remained suspended. The method is called diafiltration, to indicate its similarity to dialysis. The diafiltered cloud obtained in this way from the concentrate did not seem to be greatly changed from its natural state. Its stability, evaluated by the standard method of centrifuging at low speed and measuring the optical density of the supernatant, was equal to that of normal juice. This suggests that the cloud is not stabilized by soluble factors in juice.

A dye-binding method was developed as a tool for monitoring changes in surface protein of the cloud. The dye, amido black, binds specifically to proteins and apparently is unable to penetrate below the surface of the cloud particles. In practice, an excess of the dye was added to a juice sample, which was then centrifuged at high speed to completely separate the cloud. The amount of dye remaining in the supernatant was determined by reading the optical density, and subtraction of this value from the total dye added gave

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*Work supported in part by the Lemon Products Technical Committee.

the amount bound to the cloud. As an example of the application of this method, reconstituted juice concentrate was found to bind 36% more dye than fresh juice. This is consistent with the fact that concentrate cloud particles are smaller, and therefore have a larger surface area.

When juice was subjected to ultrasonic radiation, in an attempt to decrease the size of the cloud particles, aggregation and destabilization of the cloud occurred. Apparently, sonic disruption of the cloud particles exposes fresh surfaces which, because of their electrical charge or chemical nature, tend to adhere to other particles. Since diafiltered juice was also destabilized by sonication, soluble factors in the juice do not seem to be involved.

LEMON JUICE CLOUD: RECENT EXPERIENCE WITH PARTICLE SIZE DISTRIBUTIONS*

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The incessant motion of molecules plays an important role in the coagulation of aerosols and aquasols. A variety of sols that have been discussed in the literature coagulate according to established theory in which the rate is diffusion limited whereas lemon juice cloud coagulates much more slowly than the theory suggests. The comparatively high stability of the lemon juice particle suspension indicates the presence of sufficient interparticle repulsion to render most collisions (or near collisions) ineffective in terms of establishing permanent contact. The existence of repulsive forces that are large enough to render cloud suspensions comparatively stable does not imply, however, that the particles are inherently incapable of adhering to each other. That they can adhere firmly is easily shown with a centrifuge. When we applied a wide range of sedimentation forces to samples of fresh unpasteurized juice, the resulting pellets invariably displayed appreciable mechanical strength. The strength of these pellets reveals the existence of a significant potential for firm particle-to-particle adhesion.

The foregoing observations help to emphasize the desirability of inquiring into the nature of the coagulation barrier that evidently exists. A recent study carried out, using silver iodide sols (Melville, Willis, and Smith, J. Chem. Soc. Faraday Trans. 1 68(3):450-455, 1972), suggested a plausible approach. In this work the sols were pelleted by centrifugation and then subjected to mild, controlled agitation. The amount of sol that was redispersed in this way was affected not only by sedimentation force but also by changes in interparticle repulsion brought about by altering the composition of the suspending electrolyte. The results of the silver iodide experiments were used to draw conclusions concerning the nature of the forces that govern coagulation behavior.

We made a test of the applicability of the approach of Melville and coworkers using fresh, unpasteurized juice samples. The mechanical strength of the lemon juice pellets referred to earlier ruled out the use of the mild redispersion technique that was successfully applied in the silver iodide work. In attempting to overcome the problem posed by tenacious particle-to-particle adhesion, we completely redispersed pellets in an all-glass tissue homogenizer using the least possible number of pestle traverses. When the resulting suspensions were compared with an untreated control, the behavior was found to be somewhat complex, i.e., a plot that might have been expected to show the advancement of agglomeration with increasing sedimentation force appeared to have a distinct minimum. Such a minimum did not occur in the work

*Supported in part by the Lemon Products Technical Committee.

with silver iodide sols. We tentatively assume that the existence of the minimum reflects the comparative ease with which some of the lemon juice particles can be disrupted by hydraulic shear.

The results we obtained by sedimenting lemon juice particles and then attempting to redisperse them help in perceiving the interrelationship of such cloud properties as pellet mechanical strength, particle cohesiveness, and particle fragility. Such increased acquaintance with particle properties should provide useful guidance in the planning of new experiments.

ANALYSIS OF SOME ORANGE FLAVOR FRACTIONS AND TASTE EVALUATION OF SOME ORANGE FLAVOR COMPONENTS

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and

E. M. Ahmed
University of Florida
Gainesville, Florida

Several natural orange flavor fractions have been quantitatively and qualitatively analyzed to try to better define the components needed for a good orange flavor. Fractions analyzed included several cold-pressed orange oils and a highly volatile fraction from cold-pressed orange oil.

Cold-pressed orange oil was analyzed by gas chromatography on a non-polar column, and the 17 main components were identified and quantitated. A synthetic mixture of 15 of these components was prepared in the proper proportion as indicated by the analytical results, and was evaluated in flavor studies discussed below.

A highly volatile fraction with essencelike aroma was separated from cold-pressed orange oil and analyzed. Ten components were identified, including two, isoprene and 3-methyl-1-butene, not previously reported in orange oil. Taste panel studies showed that this volatile fraction was important to aroma and flavor of cold-pressed orange oil when used to flavor orange juice.

From these and earlier reported studies on orange flavor fractions, certain components were selected as being important contributors to orange flavor. Sensory evaluation methods were used to compare the flavor of these selected orange oil and essence components added to pumpout orange juice with that of reference (good quality) orange juice.

Three modified pumpout samples and two reference juices were presented to a group of 11 trained panelists. The two reference juices consisted of a pumpout juice assigned a rating of 1 and a good quality juice assigned a rating of 10. Panelists were asked to smell and taste each sample and assign a rating for its flavor as compared to the two known reference samples. Panel response could be summarized as follows: (a) pumpout orange juice received a rating of 2.5 (30% of maximum rating) while reference orange juice received the rating of 8.4 (100% of maximum); (b) mixtures of compounds containing acetaldehyde, citral, ethyl butyrate, d-limonene, nonanal, octanal, and α-pinene added to pumpout orange juice resulted in improving the rating of pumpout juice from 30% to 75-83% of maximum rating; (c) the highest ratings were obtained from the mixtures of: citral, ethyl butyrate, and d-limonene (79%); acetaldehyde, citral, ethyl butyrate and d-limonene (81%); citral, ethyl butyrate, d-limonene, and α-pinene (82%); and ethyl butyrate, d-limonene, and nonanal

(83%), and (d) addition of either decanal, citronellal, or trans-2-hexanal lowered the ratings of the modified pumpout juice.

Sixty-two untrained panelists, ranging in age from 20 to 60 years old and representing different sexes and races, were presented two samples of pumpout orange juice containing different combinations of the selected components and one sample of a good quality (reference) orange juice. They were asked to indicate which modified pumpout juice was closer in flavor to the reference juice.

Samples containing acetaldehyde, ethyl butyrate, d-limonene, and octanal; citral, ethyl butyrate, d-limonene, and α-pinene; and acetaldehyde, citral, ethyl butyrate, d-limonene, and octanal received the highest ratings by the untrained panel.

It seems that pumpout juice containing acetaldehyde, citral, ethyl butyrate, d-limonene, and octanal received the highest rating by the methods of sensory panels used.

LIMONIN CONTENT OF JUICE FROM MARRS AND HAMLIN EARLY ORANGES FROM SOUTH TEXAS

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Food Crops Utilization Research Unit
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During the past decade considerable plantings of the Marrs early orange have come into production in south Texas. Although the Marrs is primarily produced for the fresh fruit market, within recent years greater volumes of Marrs fruit are being processed into single strength juice and concentrate.

The development of delayed limonin bitterness was considered to be a likely potential problem with Marrs processed juice for two reasons:

First: The Marrs variety developed as a bud sport of the Washington Navel, a variety with a delayed limonin bitterness problem in processed juice when grown under Californian and Australian conditions of climate and root-stock preference.

Second: Marrs is an extremely early variety, harvest often beginning in early September. Delayed limonin bitterness is known to be more pronounced in juice from early season fruit.

Because of these considerations, we conducted a survey of the limonin content which developed in the juice of Marrs and Hamlin early oranges grown in south Texas.

At 2-week intervals beginning in September, 30 fruit, randomly selected from each of five groves distributed over the south Texas citrus producing area, were juiced on an FMC Model 091B in-line test extractor. The juice samples were deaerated, heated, and, after 2 days storage, were frozen until analyzed.

Analyses for limonin were performed by the TLC method developed by Tatum and Berry (J. Food Sci. 38:1244-1246, 1973). In attempts to automate the quantitation of the limonin on the TLC plates by use of scanning reflectance spectrophotometry or fluorescence, the instrument was found to lack the required sensitivity which the human eye was capable of providing.

Limonin content of the juice of both varieties showed only subtle and sometimes inconsistent differences between the five groves at the same harvest dates.

The data show that after reaching maturity neither variety yielded juice with limonin concentrations sufficient to produce undesirable bitterness. By the end of the early orange season, limonin content had decreased to an insignificant level.

The following year similar samplings for the two varieties from a single grove were obtained at monthly intervals and analyzed as before for limonin. Both raw and pasteurized juice from the same harvest date were found to have

no significant difference in limonin content between them and were similar to the prior years results for the comparable date.

From these analyses, we conclude that under south Texas growing conditions Marrs and Hamlin early oranges are unlikely to yield processed juice with undesirable bitterness.

A PERSPECTIVE OF LIMONOID METABOLISM AND ENZYMATIC APPROACHES TO DEBITTERING OF CITRUS JUICES*

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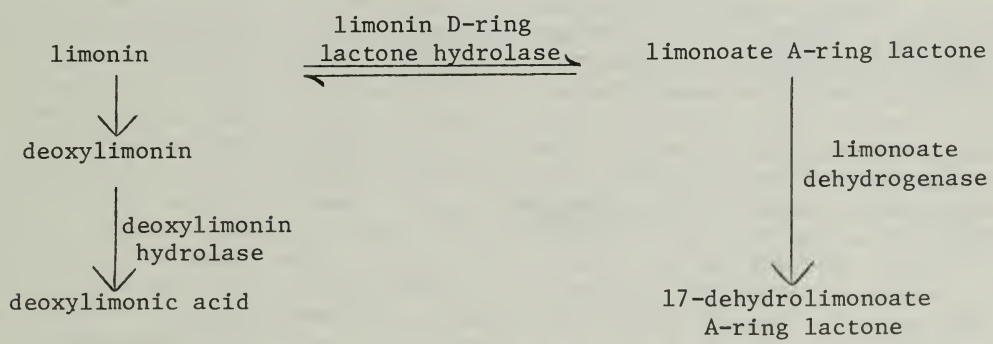
Pasadena, California

Several years ago we set out to find microorganisms that metabolize limonoids. One goal of this search was to isolate an enzyme which could be used to debitter citrus juices having limonin bitterness. Concomitant to that purpose was the hope of learning more about limonoid metabolism. Work has been progressing on the metabolism of limonoids in citrus fruit tissues. Microorganisms offer a more easily manipulated system for the study of limonoid metabolism, and subsequent work has demonstrated that similar pathways occur in microorganisms and in citrus fruit.

A microbial screening program has led to the successful isolation of a few species with limonoid-metabolizing capabilities and such work is continuing. At this time, two bacteria have been isolated, identified, and characterized, namely Arthrobacter globiformis and Pseudomonas sp. 321-18. Studies of these organisms have led to the discovery of two distinct metabolic pathways. Three of the enzymes involved in these pathways have been identified.



The pathway common to both organisms is one in which limonoate, or limonoate A-ring lactone, is converted to 17-dehydrolimonoate, or 17-dehydrolimonoate A-ring lactone, by the action of limonoate dehydrogenase enzymes. In Pseudomonas sp. 321-18 another pathway exists which converts limonin to deoxylimonin and deoxylimonic acid. One of the enzymes involved in this pathway, deoxylimonin hydrolase, has been isolated. The enzyme limonin D-ring lactone hydrolase, which catalyzes the reversible conversion of limonoate to limonin, has been demonstrated in Pseudomonas.

Based on knowledge of the microbial pathways, the metabolic pathway from limonoate A-ring lactone to 17-dehydrolimonoate A-ring lactone has also been

*Work supported in part by the Lemon Products Technical Committee.

demonstrated in citrus fruit. Limonoate dehydrogenase activity has recently been demonstrated in navel orange albedo and the metabolic product of this enzyme, 17-dehydrolimonoate A-ring lactone, has been isolated from several citrus tissues. Limonin D-ring lactone hydrolase has been demonstrated in citrus tissues and isolated from citrus seeds. The existence of the metabolic pathway to deoxylimonic acid is currently being investigated.

The isolation of the two limonoate dehydrogenase enzymes from Arthrobacter globiformis and Pseudomonas sp. 321-18 opened the possibility of an enzymatic solution of the limonin bitterness problem in citrus juices. Both enzymes catalyze the same reaction, namely, the conversion of limonoate A-ring lactone to 17-dehydrolimonoate A-ring lactone, a stable, nonbitter product. The two enzymes have markedly different properties in model systems. Studies of the action of these two enzymes in navel orange juice revealed that while the limonoate dehydrogenase of A. globiformis retained only slight activity at the low pH of citrus juices, the dehydrogenase of Pseudomonas retained substantial activity. Further work with the limonoate dehydrogenase of Pseudomonas sp. 321-18 demonstrated that it can be used to effectively reduce the limonin content of freshly prepared navel orange juice to a nonbitter level. Work is progressing on the detailed properties of this enzyme.

To further explore the potential utility of the limonoate dehydrogenase of Pseudomonas sp. 321-18, work was initiated on growth of the organism and production of its limonoate dehydrogenase. The bacterium will grow on limonoate as its sole carbon source and will produce limonoate dehydrogenase from this growth medium. The organism will also grow on a number of other substrates such as citrate and various sugars. Limonoate dehydrogenase is an induced rather than a constitutive enzyme, consequently limonoate is needed for limonoate dehydrogenase production to occur. The enzyme can be induced by first growing the organism on a substrate such as galactose, and later adding a small quantity of limonoate or a limonoate containing source after substantial growth has occurred. The relationship between growth and enzyme production is currently being investigated to maximize the enzyme activity per cell as well as the total enzyme yield per unit volume of culture.

The search for new limonoid-degrading microorganisms and new enzymes is continuing aided bv our knowledge of the presently known limonoid pathways. The deoxylimonin pathway of Pseudomonas suggests that an enzyme exists which is capable of attacking limonin itself rather than limonoate A-ring lactone. Such an enzyme would offer a decided advantage over the limonoate dehydrogenase enzymes currently available because it could be used to treat juice after it had been heated or stored rather than immediately after juicing. Several new microorganisms which metabolize limonoids have recently been isolated and are being studied.

NEW FLAVONOIDS FROM KUMQUATS

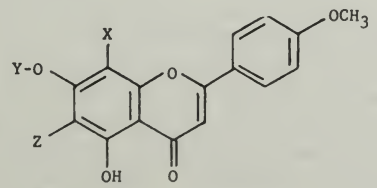
R. M. Horowitz and Bruno Gentili
Fruit and Vegetable Chemistry Laboratory
Pasadena, California

The peculiar sweetness of the albedo portion of the kumquat (Fortunella margarita) suggests the presence of a sweet principle other than the usual sugars. To test this possibility we examined the peel of the oval (Nagami) kumquat. Through crystallization or chromatography we have demonstrated the occurrence of the known flavone fortunellin; the flavanones poncirin and naringin; sucrose and glucose; three new compounds that appear to be dihydrochalcones; and two new flavones which we have named margariten and isomargariten.

Chemical, chromatographic, and ultraviolet data indicated that margariten and isomargariten are closely related to each other, that they contain a C-linked rhamnoglucosyl residue, and that the aglycone moiety is 5,7-dihydroxy-4'-methoxyflavone (acacetin). Circular dichroism data showed that the disaccharyl residue is attached through C-8 in margariten and C-6 in isomargariten, while nuclear magnetic resonance data showed that in each compound the rhamnose is attached through the C-2 hydroxy group of the glucosyl residue. Margariten and isomargariten are therefore 8-C- and 6-C-β-neohesperidosylacacetin, respectively.

Fortunellin was shown earlier to be 7-O-β-neohesperidosylacacetin. Margariten, isomargariten, and fortunellin, whose structures are shown below, constitute an unusual example of the co-occurrence of a flavone 7-O-glycoside and its carbon-linked C-6 and C-8 analogs. Taxonomic implications of these findings will be discussed.

The three presumed dihydrochalcones from kumquats have not been obtained in crystalline form, although the major compound is chromatographically pure. Based on spectral work, which will be reviewed, the aglycone portion of this compound appears to be naringenin dihydrochalcone (phloretin) to which are attached two carbon-linked sugars. If further work substantiates that these constituents of an innocuous foodstuff, kumquats, are dihydrochalcones, this will provide strong corroborative evidence for lack of toxicity of this class of compounds and could have a bearing on obtaining clearance for the dihydrochalcone sweeteners.



Margariten: X = β-neohesperidosyl; Y = Z = H
Isomargariten: Z = β-neohesperidosyl; X = Y = H
Fortunellin: Y = β-neohesperidosyl; X = Z = H

THE AUTOMATED DETERMINATION OF SEVERAL CLASSES OF JUICE CONSTITUENTS, AND THE DATA-RATIO APPROACH TO CHARACTERIZING ORANGE JUICE PRODUCTS*

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There is an urgent need by governmental regulatory agencies and the citrus industry to be able to detect adulterations of orange juice and also to determine the juice content of orange juice products. Any solution to this problem will involve the analyses of many orange juice samples for a number of constituents to establish a broad statistical data base. In order to speed up and simplify the acquisition of data, an automated analytical system was devised whereby the total sugars, reducing sugars, total acidity, total amino acids, and phenolics can be measured on a single manifold. The sample preparation and conversion from one analysis to another are quite simple.

The analysis of total acidity is quite comparable for the manual and automated procedures. The overall averages were 13.7 and 13.8 meq/100ml, respectively. The correlation coefficient was 0.979. The correlation between the Brix measurement and total sugars (r=0.792) was lower than that for the acidity measurements, because the sugar determination is much more specific for sugars than is refractive index.

The automated colorimetric method for amino acids is highly correlated with the formol titration (r=0.957), but the results are higher for the automated method since the formol reaction does not occur with proline. The increase with the ninhydrin procedure is in the range of the proline content (0.5 meq/100ml). The 485 nm wave length chosen for the reaction gave the least variation in molar color yield for the individual amino acids. The color yield effect was further minimized by selecting a standard with the average juice amino acid composition. In terms of accuracy, the colorimetric procedure has maximum error of less than 5% based on 3.0 meq/100ml amino acid mixed standards that represent the extremes in orange juice amino acid composition.

The manual and automated methods of estimating total phenolics are the least correlated (r=0.419). This is partly true because they are measuring different compounds. The manual method is a useful parameter but was never intended to be specific for just phenolics. It is the sum of all compounds that have absorbance in the 320nm region measured on a 1:20 ethanol dilution of orange juice. Being nonspecific, the "total phenolics" were always reported in absorbance units. The automated method is more specific for phenolics, although it has some limitations too. The diazotized sulfanilic acid probably does not react with some of the polymethoxylated flavonoids. There is also the problem of selecting a suitable standard. Phenol was chosen because it is

*Work supported in part by the Lemon Products Technical Committee.

readily available. There is the problem, due to the diverse nature of the phenolics, of differing reaction rates and molar color yields. As a result of these considerations, the color produced by the diazotized sulfanilic acid and orange juice is treated as a parameter of the juice, which is related to its phenolic content. The absorbance of the reaction products is reported in terms of the concentration of phenol that has equal absorbance under identical conditions.

Data have been collected from 380 samples of California, Florida, and foreign orange concentrates. Ratios of the constituents were used to eliminate the concentration effects. Significant differences were shown in many of these ratios for different growing areas. The application of the ratio differences to classify and characterize the juices will be discussed.

THE CONCEPT OF BIOREGULATION AND ITS APPLICATION TO QUALITY IMPROVEMENT OF CITRUS FRUITS

V. P. Maier and Henry Yokoyama
Fruit and Vegetable Chemistry Laboratory
Pasadena, California

Ever since the elaboration of the molecular basis of inherited characteristics and their regulation, a potential application of these regulatory mechanisms to improve agricultural crop quality has existed.

Genes, through their control of enzyme synthesis, determine whether a plant has the capacity to synthesize a given constituent. Groups of enzymes operating in biosynthetic and metabolic pathways lead, through their catalysis of simple chemical reactions, to the formation of the multitude of constituents characteristic of a given plant species or cultivar. However, the interaction of the gene with complex regulatory mechanisms, which respond to stimuli from their environment, determines the amount of the compound made and accumulated.

The quality of citrus fruits is determined, to a large extent, by the presence of too much or too little of certain constituents or groups of constituents--too much acid, limonin, and naringin or too little sugar, carotenoid color, and terpenoid flavoring constituents (citral, nootkatone, sinensal). Therefore, it is often not the absence of genes that is critical to fruit quality. Rather, it is the quantitative expression of the genetic information that is critical, in other words, the regulation of the biosynthetic pathways.

Biosynthetic pathways of the cell are regulated by two different mechanisms: the specific regulation of enzyme synthesis at the gene level and the specific regulation of enzyme activity at the enzyme level. Both mechanisms are mediated by compounds of low molecular weight, which are either formed in the cells as intermediary metabolites or enter the cells from the environment. In our work, we call these low molecular weight mediators bioregulators.

In theory, then, control of citrus fruit composition should be possible by discovering and applying to the fruit the individual bioregulators that control specific biosynthetic pathways. Research has been underway at our laboratory for a number of years to determine whether this theory would prove possible in actual practice. We now feel that the research has progressed far enough to conclude that control of citrus fruit composition through the use of bioregulators is possible in the laboratory and should eventually prove feasible on a commercial basis.

Examples will be mentioned in this and the following papers of how we have been able to influence the composition of fruits in specific ways by postharvest application of solutions of simple, small molecule bioregulators. Details of how a small molecule can be envisioned to exert such profound influence over entire biosynthetic pathways will be discussed. Lastly, the broad application of this concept to improved crops will be summarized.

ENHANCEMENT OF COLOR AND PROVITAMIN A QUALITY OF CITRUS FRUIT*
I. RECENT DEVELOPMENTS IN STUDIES ON BIOREGULATORS OF CITRUS COLOR FORMATION

Henry Yokoyama, Wan-Jean Hsu, Stephen M. Poling, and Charles DeBenedict
Fruit and Vegetable Chemistry Laboratory
Pasadena, California

In our work relative to the induction of desirable color and provitamin A responses in oranges and other citrus fruits, we have developed a large number of bioregulators. These bioregulators have been classified into three groups according to the carotenoid pattern observed. Group I bioregulators causes a rapid and extensive accumulation of the red carotenoid pigment lycopene and, to a lesser extent, the provitamin A γ-carotene. With Group II bioregulators, citrus fruit color goes rapidly to deep orange and then continues on to red, accompanied by a significant increase in provitamin A α-, β- and γ-carotenes. Group III bioregulators are the first agents found that produce a substantial increase in orange-colored carotenoids in citrus fruits but form only insignificant amounts of the red lycopene. Fruit color development, therefore, never goes beyond the deep-orange stage.

Within the past year, we have developed a number of new bioregulators in Groups II and III which produce highly desirable color and provitamin A responses in citrus fruits. A series of new bioregulators in Group II causes the stimulation and accumulation of β-carotene. As reported last year, we had observed a twofold to fivefold increase in the total provitamin A carotenoid content. Now we report up to a twentyfold increase in the amount of β-carotene alone. β-carotene is a most desirable carotenoid pigment because of its deep-orange color and high provitamin A properties. Further studies on the mode of action of these new bioregulators are continuing.

A new Group III bioregulator was synthesized which stimulates the production and accumulation of the overall carotenoid content in citrus fruits. As in the case with other Group III bioregulators previously reported at this meeting, fruit color development never goes beyond the deep-orange stage. Further work is underway to study the nature and details of the stimulatory effect of this new bioregulator on overall carotenoid formation. The implications of these recent new developments will be discussed.

In the design and synthesis of bioregulators, certain factors are taken into consideration in the search for greater effectiveness and improved properties. These will be described in detail in the following two papers.

*Supported in part by the California Citrus Advisory Board and the Florida Citrus Commission.

II. STRUCTURE - ACTIVITY RELATIONSHIP: CORRELATION OF ACTIVITY TO VALUE OF LOG PARTITION COEFFICIENT

Stephen M. Poling, Wan-Jean Hsu, and Henry Yokoyama
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Pasadena, California

The ability of CPTA and other derivatives of triethylamine to enhance the natural color and provitamin A content of citrus has been previously reported. These bioregulators stimulate the natural biosynthetic pathway causing an increased accumulation of the naturally occurring carotenoid pigments and therefore an enhanced color and provitamin A content. In an effort to determine the relationship between the structure of the bioregulators and their ability to stimulate the accumulation of pigments, 15 new compounds were synthesized and tested on Marsh seedless grapefruit. The compounds fall into three series: $Et_2N(CH_2)_nCH_3$(n=4-8), $Et_2N(CH_2)_nPh$(n=1-5), and $Et_2NCH_2CH_2OC_6H_4R$ (R=H, p-Me, p-Et, p-iso-Pr, p-tert-Bu). These bioregulators caused up to an elevenfold increase in the pigment content.

The Marsh seedless grapefruit was used in these tests because of the relative simplicity of the carotenoid pattern and the ease of detecting any color changes visually. The compounds are equally as effective on oranges. The compounds were applied as either the free amine or the hydrochloride in isopropanol.

Examination of the flavedo of the treated fruit showed that the response pattern was similar to that of fruit treated with CPTA. The main effect was to stimulate the accumulation of the red pigment, lycopene. There were also significant increases in the amount of ζ-carotene. Two of the compounds, $Et_2N(CH_2)_7CH_3$ and $Et_2N(CH_2)_8CH_3$, caused 3.5- and 4.6-fold increases, respectively, in the provitamin A carotenoids.

The ability of these bioregulators to stimulate biosynthesis shows a strong correlation to the value of the logarithm of the octanol-water partition coefficient (log P). The partition coefficient is the concentration of the compound in octanol divided by the concentration in water when the two phases are in contact and represents the relative affinity of the compound for lipids as compared to aqueous solutions. Log P will reflect the ability of the compound to pass through the various aqueous and lipid layers in the cell and therefore bears a strong correlation to the concentration at the regulatory site.

Like CPTA, some of these compounds cause the fruit to change rapidly from the natural yellow color to pink or red due to lycopene accumulation. Other of the bioregulators cause a slower color development, with the fruit becoming a uniform orange color, which is retained for several weeks and eventually becoming pink or red.

These new compounds show a potential usefulness for increasing the nutritional level, by increasing provitamin A carotenoids, as well as in bringing about desirable color changes in citrus fruit.

III. STRUCTURE - ACTIVITY RELATIONSHIP: CONSIDERATION OF LOG PARTITION COEFFICIENT, ELECTRONIC, AND STERIC FACTORS

Wan-Jean Hsu, Stephen M. Poling, Charles DeBenedict, and Henry Yokoyama
Fruit and Vegetable Chemistry Laboratory
Pasadena, California

The compound CPTA and its oxy analog were found to have a profound effect on the induction of lycopene formation in a wide array of carotenogenic tissues including citrus fruits. In order to determine the essential structural features for inducer activity and to see how these influence the effectiveness of the inducers, a series of triethylamine derivatives were prepared and tested for inducer activity on Marsh white grapefruit (Citrus paradisi, Rutaceae). They were all found to be effective, but the degree of effectiveness differed.

The structural feature essential for inducer activity is the tertiary alkyl amine group. Compounds with an S-atom joining the amine portion and the benzene moiety are more effective than their oxy-analogs, whereas benzene derivatives are more effective than alkyl derivatives.

In the oxy-series of chemical inducers, 2-[4-chlorophenoxy]-triethylamine was found to be much more effective than 2-phenoxytriethylamine. Effort was then made to investigate whether the inducer activity of amine compounds is parallel to the electronic characteristics of the substituting groups on the benzene moiety. Test results showed that the inducer activity of the compound can be enhanced by introducing a strong electron-withdrawing group into the molecule; however, the effectiveness of the compounds does not depend solely upon the electron-withdrawing ability of the substituting group.

The variable effectiveness on carotenogenesis in flavedo tissue among the triethylamine derivatives suggests the possible existence of a penetration problem. The Hansch approach was thus considered. In general, the relationship observed between effectiveness and log partition coefficient (log P) in the same series of compounds seemed to indicate that the higher the log P of the compound, the higher the biological activity will be. The addition of $-COCH_3$ group (σ value of +0.874) to the benzene ring of the 2-phenoxytriethylamine molecule lowers its log P value (3.05) by 0.37; however, it enhanced the activity by seventeenfold. The 2-(3-chlorophenoxy)- and 2-(2-chlorophenoxy)-triethylamines have log P values (3.81 and 3.64) similar to that of the p-isomer (3.75). However, in comparison with the reference compound, 2-phenoxytriethylamine, the p- substitution of a chlorine atom enhanced the inducer activity; substitutions at the m- and o- positions decreased it drastically. The low activities observed here might be due to the steric hindrance produced by the m- and o- substituents.

The above results indicate that the log P values of the compounds can be used as a general guide in designing the structure of new bioregulators. However, the possible electronic and steric effects exerted by the groups introduced into the molecule should also be considered. Through studies of

this sort we are developing criteria to apply in the design of the most promising bioregulators of carotenogenesis for use on citrus fruits to enhance color and provitamin A content.

ENHANCEMENT OF THE CITRAL CONTENT OF LEMONS

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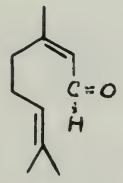
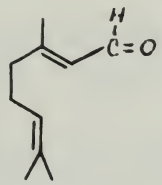
The flavor and quality of lemon oil is closely related to the total aldehyde content of the oil. The major constituent of these aldehydes is citral. Citral is composed of two isomers; neral and geranial:



neral
(citral B)



geranial
(citral A)

In these studies, the concentration of the above two compounds in the peel of lemons was used as a measure of the total aldehyde content of the oil obtainable from the lemons. Green mature lemons were treated with various bioregulators by the vacuum infiltration technique. The fruit were treated with ethylene until color break was observed and then stored for an additional week in the laboratory. Fruit were peeled, peels extracted, and levels of citral determined by gas liquid chromatography. A 2.5-fold increase in citral concentration for some treatments was observed.

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LIST OF PUBLICATIONS AND PATENTS*

WESTERN REGION

Fruit and Vegetable Chemistry Laboratory
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DIHYDROCHALCONE XYLOSIDES AND THEIR USE AS SWEETENING AGENTS. Horowitz, Robert M. and Gentili, Bruno. U.S. Patent No. 3,826,856, patented July 30, 1974.

EFFECT OF SOME CITRUS JUICE CONSTITUENTS ON TASTE THRESHOLDS FOR LIMONIN AND NARINGIN BITTERNESS. Guadagni, Dante G., Maier, Vincent P., and Turnbaugh, Jean G. J. Sci. Food Agr. 24, 1277-1288, 1973.

ISOLATION OF NEW LIMONOATE DEHYDROGENASE FROM PSEUDOMONAS. Hasegawa, Shin, Maier, V. P., and King, A. Douglas, Jr. J. Agr. Food Chem. 22(3), 523-526, 1974.

EFFECTS OF AMINES ON THE CAROTENOGENESIS IN BLAKESLEA TRISPORA. Hsu, Wan-Jean, Yokoyama, Henry, and Poling, Stephen M. Phytochem. 13, 415-419, 1974.

DETECTION OF LIMONOATE DEHYDROGENASE ACTIVITY IN ALBEDO TISSUES OF CITRUS SINENSIS. Hasegawa, Shin, Maier, V. P., and Bennett, Raymond D. Phytochem. 13, 103-105, 1974.

VITAMIN INDUCTION IN FRUITS AND VEGETABLES WITH BIOREGULATORS. Maier, V. P. and Yokoyama, Henry. Nutritional Qualities of Fresh Fruits and Vegetables, White, P. L. and Selvey, N., eds., Futura Publishing Co., 177-180, 1974.

CITRUS BITTER PRINCIPLES. XII. PHOTOCHEMISTRY OF LIMONIN. Dreyer, David L. and Rigod, Jean F. J. Org. Chem. 39(2), 263-265, 1974.

RAPID DETERMINATION OF γ-AMINOBUTYRIC ACID AND ARGININE IN ORANGE JUICE: APPLICATION TO DETECTING ADULTERATION. Vandercook, Carl E. and Price, Ruth L. JAOAC 57(1), 124-129, 1974.

USE OF LIMONOATE DEHYDROGENASE OF ARTHROBACTER GLOBIFORMIS FOR THE PREVENTION OR REMOVAL OF LIMONIN BITTERNESS. Hasegawa, Shin, Brewster, Linda C., and Maier, V. P. J. Food Sci. 38, 1153-1155, 1973.

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*Reprints are available at the addresses indicated; patents are available only by purchase at 50¢ a copy from the U.S. Patent Office, Washington, D.C. 20231.

LEMON JUICE PARTICULATES: COMPARISON OF SOME FRESH JUICES AND A COMMERCIAL CONCENTRATE. Venolia, Wayne, Peak, Shirley, and Payne, Fred. J. Agr. Food Chem. 22(1), 133-136, 1974.

DIHYDROCHALCONE SWEETENERS. Horowitz, Robert M. and Gentili, Bruno. Symposium: Sweeteners, Inglett, G. E., ed., AVI Publishing Co., 182-193, 1974.

CAROTENOGENESIS IN PHYCOMYCES. Hsu, Wan-Jean, Ailion, D. A., and Delbruck, Max. Phytochem. 13, 1463-1468, 1974.

Western Regional Research Center
Berkeley, California 94710

ULTRAVIOLET ABSORPTION METHOD FOR EVALUATING CITRUS ESSENCES. Randall, J. M., Bryan, W. L., Bissett, O. W., and Berry, R. E. J. Food Sci. 38, 1047-1049, 1973.

EFFECT OF SOME CITRUS JUICE CONSTITUENTS ON TASTE THRESHOLDS FOR LIMONIN AND NARINGIN BITTERNESS. Guadagni, Dante G., Maier, Vincent P., and Turnbaugh, Jean G. J. Sci. Food Agr. 24, 1277-1288, 1973.

DIHYDROCHALCONE SWEETENERS: PREPARATION OF NEOHESPERIDIN DIHYDROCHALCONE. Robertson, George H., Clark, J. Peter, and Lundin, Robert. I&EC Product Research and Development 13, 125-129, 1974.

SOUTHERN REGION

Citrus and Subtropical Products Laboratory
600 Avenue S, N.W., Winter Haven, Florida 33880

PROTEASE AND PECTINASE ADDITIVE TO CITRUS JUICES. Baker, Robert A. and Bruemmer, Joseph H. U.S. Patent No. 3,754,932, patented August 28, 1973.

INFLUENCE OF SELECTED 5'-NUCLEOTIDES ON FLAVOR THRESHOLD OF OCTANAL. Schinneller, D. J., Dougherty, R. H., and Biggs, R. H. J. Food Sci. 37, 935-937, 1972.

METHOD FOR ESTIMATING LIMONIN CONTENT OF CITRUS JUICES. Tatum, James H. and Berry, Robert E., J. Food Sci. 38, 1244-1246, 1973.

IMPROVED COLORIMETRIC DETERMINATION FOR FURFURAL IN CITRUS JUICES. Dinsmore, Howard L. and Nagy, Steven. JAOAC 57(2), 332-335, 1974.

POTENTIAL BY-PRODUCTS FROM WASTE CITRUS PEEL EMULSION. Bryan, W. L., Bissett, O. W., Wagner, C. J., Jr., and Berry, R. E. Proc. Fla. State Hort. Soc. 86, 275-280, 1973.

OILS RECOVERED FROM CELERY PACKINGHOUSE WASTE. Lund, E. D., Wagner, C. J., Jr., and Bryan, W. L. Proc. Fla. State Hort. Soc. 86, 255-259, 1973.

FATTY ACID COMPOSITION OF STEROL ESTERS FROM CITRUS SINENSIS, C. PARADISI, C. LIMON AURANTIFOLIA AND C. LIMETTIOIDES SACS. Nordby, Harold E. and Nagy, Steven. Phytochem. 13, 443-452, 1974.

ENZYME MEDIATED ALDEHYDE CHANGE IN ORANGE JUICE. Roe, Bongwoo and Bruemmer, Joseph H. J. Agr. Food Chem. 22(2), 285-288, 1974.

QUANTITATIVE AND QUALITATIVE ANALYSIS OF TANGERINE PEEL OIL. Moshonas, Manuel G. and Shaw, Philip E. J. Agr. Food Chem. 22(2), 282-284, 1974.

PROGRESS ON MECHANICALLY GRADING CITRUS FRUIT IN PROCESSING PLANTS. Bryan, William L. Transactions of the 1974 Citrus Eng. Conference, Vol. XX, 24-35, 1974.

POTENTIAL USES OF DISTILLED ORANGE OILS. Coleman, Richard L. and Shaw, Philip E. The Citrus Industry 55(2), 20-21, 1974.

FATTY ACIDS OF TRIGLYCERIDES FROM CITRUS JUICE SACS. Nagy, Steven and Nordby, Harold E. Phytochem. 13, 153-157, 1974.

Food Crops Utilization Unit
P. O. Box 388, Weslaco, Texas 78596

JUICE QUALITY OF RUBY RED GRAPEFRUIT AS AFFECTED BY VARIABLE AMOUNTS OF PETROLEUM SPRAY OIL APPLIED AT POSTBLOOM. Dean, H. A., Lime, Bruce J., and Cruse, Robert R. J. Rio Grande Valley Hort. Soc. 26, 7-12, 1972.

APPLICATIONS OF THE CITRUS COLORIMETER TO TEXAS ORANGE JUICE. Cruse, R. R. and Lime, Bruce J. J. Rio Grande Valley Hort. Soc. 28, in press, 1974.

CHANGES IN LYCOPENE-CAROTENE CONTENT AND FRUIT QUALITY OF HUDSON RED GRAPEFRUIT. Meredith, F. J., Redman, G. H., and Griffiths, F. P. J. Rio Grande Valley Hort. Soc. 28, in press, 1974.

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